year round in the laboratory on moist cotton seeds. The experimental insects were sorted out, marked, and kept in separate bottles. Cotton swabs soaked with amino acid solutions were given to the insects daily. The experiments were conducted in 3 sets.

Insects were fed on: 1. a) Essential amino acids (argenine, histidine, lysine, tryptophane, phenylalanine, methionine, threonine, leucine, isoleucine, valine). b) Non-essential amino acids (serine, α-alanine, β-alamine, glutamic acid, glycine, aspartic acid, tyrosine, cystine, proline, hydroxyproline). c) All 20 amino acids. 2. All essential and one non-essential amino acid. 3. All amino acids were given omitting in each experiment a different one of the essential amino acids. The results reported were checked in 3 to 5 series of observations.

Results. In the first experiment nymphs kept in 3 separate bottles were provided either with the essential amino acids, the non-essential amino acids or all the 20 amino acids. The nymphs fed on all 20 amino acids grew and moulted to the adult stage, while in the other 2 bottles they died within 3 to 7 days.

In the second set of experiments, the insects were provided with the 10 essential plus 1 of the non-essential amino acids. In these combinations the nymphs died before reaching the maturation stage, except in the presence of glutamic acid, glycine, and aspartic acid.

In the 3rd set of experiments the omission test was applied, i.e. all amino acids were given but for the omission of one of each of the essential amino acid. The omission of each one of the essential amino acids stopped growth and moulting, except when phenylalanine was omitted and tyrosine was given in place of phenylalanine. However, tyrosine could replace phenylalanine only when added in higher quantity; otherwise the insects died before reaching maturity. In the absence of phenylalanine, a few insects survived and reached maturity to some extent.

Discussion. Dysdercus reared only on essential amino acids or on non-essential amino acids could not reach maturity. These observations are in accordance with the results of previous studies on amino acid requirements of insects, which generally grow poorly or not at all with only the essential amino acids provided, even when these are given at higher concentrations, as was noted in the silk worm *Bombyx mori*[10], the aphid *Myzus persicae*[6] and many others. Exceptions to this include the flour beetle *Tribolium confusum*[11], the boll weevil *Anthonomos grandis*[12] and the red banded leaf roller *Argyrotaenia velutinana*[4].

When the essential amino acids were supplemented with aspartic acid, glutamic acid or glycine, growth and moulting of *D. similis* was found to be normal, similar to the situation in *Bombyx mori*, where optimal growth occurred when the essential amino acids were supplemented with either aspartic or glutamic acid[10]. Likewise, cystine was found to be an essential component for *Aedes*[13] and glycine for *Pseudosarcophaga*[14]. In the present study, either glutamic acid, glycine or aspartic acid were found to be indispensable for optimal growth and development in *D. similis*.

It has been observed in many insect species that there exist unusual synthetic mechanisms: for instance, the ability of *Phormia* to use cystine in place of methionine[15], and of *Aedes* to use tyrosine in place of phenylalanine[13], though according to SINGH and BROWN[16], tyrosine cannot replace phenylalanine in *Aedes aegypti*. In the present study, it has been observed that in *D. similis* phenylalanine can be replaced by tyrosine.

[1] The author is grateful to Prof. R. S. SAINI for his guidance, critical discussions and encouragement.

[2] H. L. HOUSE, in *The Physiology of Insecta*, 1st edn. (Ed. M. ROCKSTEIN; Academic Press, New York and London 1965), vol. 2, p. 769.
[3] N. C. PANT, P. GUPTA and J. K. NAYAR, Experientia *16*, 311 (1960).
[4] G. C. ROCK and K. W. KING, J. Insect Physiol. *13*, 59 (1967).
[5] T. ITO and N. ARAI, Bull. seric. Exp. Stn. Japan. *19*, 345 (1965).
[6] R. H. DADD and D. L. KRIEGER, J. Insect Physiol. *14*, 741 (1968).
[7] H. LIPKE and G. FRAENKEL, A. Rev. Entom. *1*, 17 (1954).
[8] D. GILMOUR, *Biochemistry of Insects* (Academic Press, London 1961), p. 6.
[9] H. L. HOUSE, A. Rev. Entom. *6*, 13 (1961).
[10] T. ITO and N. ARAI, J. Insect Physiol. *12*, 861 (1966).
[11] A. F. NAYLOR, Can. J. Zool. *41*, 1127 (1963).
[12] E. S. VANDERZANT, J. Insect. Physiol. *11*, 659 (1965).
[13] L. GOLDBERG and B. DEMEILLON, Biochem. J. *43*, 372 (1948).
[14] H. L. HOUSE, Can. J. Zool. *32*, 351 (1954).
[15] E. HODGSON, V. H. CHELDELIN and R. W. NEWBURGH, Can. J. Zool. *34*, 527 (1956).
[16] K. R. P. SINGH and A. W. A. BROWN, J. Insect Physiol. *1*, 199 (1957).

Production of β-Ergokryptine by a Strain of *Claviceps purpurea* (Fr.) Tul. in Submerged Culture

M. BIANCHI, A. MINGHETTI and C. SPALLA

Farmitalia S.A., Viale Bezzi 24, C.P. 3075, I–20146 Milano (Italy), 21 July 1975.

Summary. A strain of *Claviceps purpurea*, labelled 231 F.I., produced in submerged culture 1200 μg/ml of a mixture of peptidic alkaloids composed for 30% by β-ergokryptine. Cultural media, conditions of culture and development features of a typic fermentation are reported.

The ergot alkaloid β-ergokryptine was isolated in 1967 from sclerotia[1]. While it is well known that several ergot alkaloids can be produced by strains of *Claviceps* in submerged cultures[2,3], the production of β-ergokryptine in these conditions has never been reported.

In the course of an investigation of strains isolated from sclerotia of *Claviceps purpurea*, a strain able to produce β-ergokryptine in submerged culture was obtained. The strain, labelled 231 F.I., was grown on slants of medium T2 at 28 °C for 8 days and then transferred into 300-ml Erlenmeyer flasks containing 50 ml of the inoculum medium TG. The flasks were incubated 4 days at 24 °C on a rotary shaker operating at 225 rpm with a 3 cm

[1] W. SCHLIENTZ, R. BRUNNER, A. RÜEGGER, B. BERDE, E. STÜRMER and A. HOFMANN, Experientia *23*, 991 (1967).
[2] A. M. AMICI, A. MINGHETTI, T. SCOTTI, C. SPALLA and L. TOGNOLI, Experientia *22*, 415 (1966).
[3] A. M. AMICI, A. MINGHETTI, T. SCOTTI, C. SPALLA and L. TOGNOLI, Appl. Microbiology *18*, 464 (1969).

Composition of the culture media

Components	Nutrient agar (T2)	Inoculum medium (TG)	Production medium (T25)
Sucrose	100 g	100 g	300 g
L-asparagine	10 g	—	—
Citric acid	—	10 g	15 g
$Ca(NO_3) \cdot 4H_2O$	1 g	—	—
KH_2PO_4	0.25 g	0.5 g	0.5 g
$MgSO_4 \cdot 7H_2O$	0.25 g	0.3 g	0.25 g
Yeast extract	0.1 g	0.1 g	0.1 g
KCl	0.12 g	—	0.12 g
$FeSO_4 \cdot 7H_2O$	0.020 g	0.007 g	0.007 g
$ZnSO_4 \cdot 7H_2O$	0.015 g	0.006 g	0.006 g
Agar	20	—	—
Aqueous ammonia	—	to pH 5.2	to pH 5.2
NaOH	to pH 5.2	—	—
Tap water	to 1,000 ml	to 1,000 ml	to 1,000 ml
Sterilization	110 °C for 20 min	110 °C for 20 min	110 °C for 20 min

throw. Aliquots of the culture obtained were used to inoculate 300-ml flasks containing 30 ml of the production medium T25, which were incubated as described for the inoculum cultures. The compositions of the culture media are reported in the Table.

After 15 days the cultures contained an average of 1,200 µg/ml of peptide alkaloids. After extraction of total alkaloids, the isolation of β-ergokryptine was performed according the method described[4].

The amount of the compound corresponded to about 30% of the total alkaloids. β-ergokryptine obtained crystallized from benzene with m.p. 173° (dec); $[\alpha]_D^{20} = -174°$ (in $CHCl_3$). By hydrolysis under various conditions,

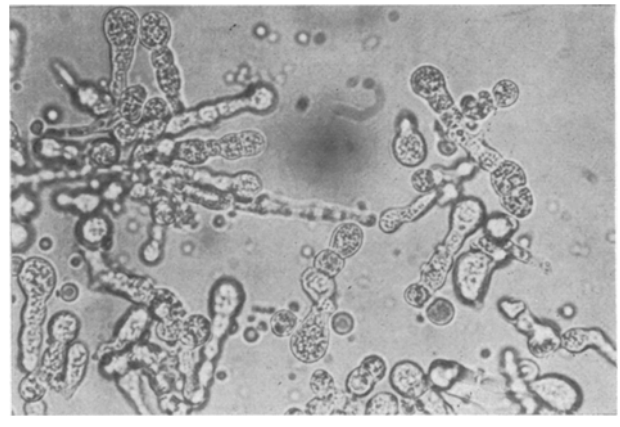

Fig. 2. Arthrosporoid-like structures in a 12-day culture of *C. purpurea* 231 F.I. in medium T25.

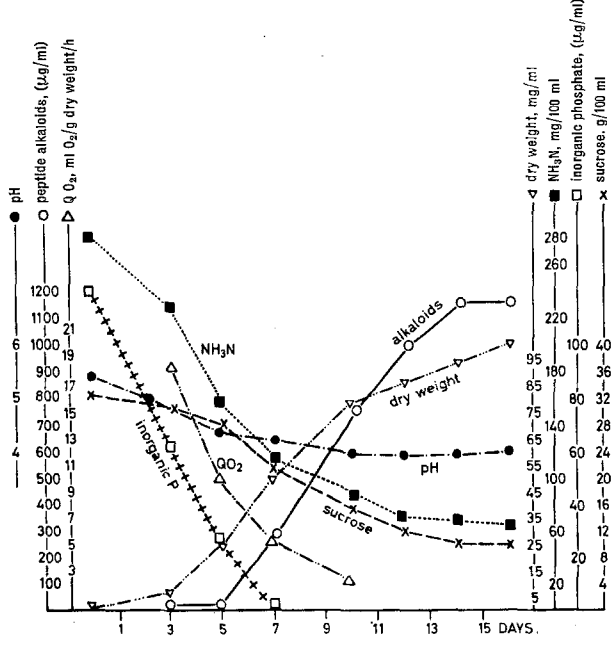

Fig. 1. Course of a typical fermentation with *C. purpurea* 231 F.I.

one equivalent of each isoleucine, proline, lysergic acid and dimethylpyruvic acid were obtained. Mass spectrometry analysis confirmed the structure.

The course of a typical fermentation is shown in Figure 1. As the fermentation progresses most hyphae swell and fragmentize into arthrosporoid-like structures, which contain large amounts of lipid granulations (Figure 2).

[4] W. SCHLIENTZ, R. BRUNNER, A. RÜEGGER, B. BERDE, E. STÜRMER and A. HOFMANN, Pharmac. Acta helv. *43*, 497 (1968).